CHANGES IN VISCOSITY OF MID-BRAIN CELL SECRETIONS AS PARAMETERS OF PROBABLE INDUCTION OF BRAIN MORPHOGENESIS IN THE CHICK EMBRYO

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WILLIE WALTER HOUSTON, JR.

DEPARTMENT OF BIOLOGY

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ABSTRACT

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HOUSTON, JR. WILLIE WALTER B.S., MOREHOUSE COLLEGE, 1974

Changes in Viscosity of Mid-Brain Cell Secretions as Parameters of Probable Induction of Brain Morphogenesis in the Chick Embryo

Advisor: Dr. John Browne

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In the past few years, results have shown that the ontogeny of embryonic fluids can be studied and that they are important to the orderly changes in cellular aggregates of the embryo that subsequently lead to defined morphogenesis. Studies have been done on normal viscosity changes in cerebrospinal fluid (CSF) of the chick embryo, using a Wells-Brookfield microviscometer, on days 4 through 8 of development. Results obtained show that there are marked changes in these extracted cell secretions from days 4 through 8, a period of rapid brain expansion and accompanying morphogenesis in the chick embryo. Moreover, dimethylsulfoxide (DMSO) was used as a source of physiological insult in the chick embryos. Results have shown that when 0.5 cc of DMSO is injected into the air-sac of the 4-day embryo, and incubation continued through day 8, there are again marked changes in these extracted cell secretions. The overall effects on brain morphogenesis, with specific emphasis on changes in viscosity changes,

serve as one parameter of physiological regulation underlying sequential development of the brain of the chick embryo.

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CHAPTER I

INTRODUCTION

The most primitive vertebrate brain and the brain of vertebrate embryos during early development consist of three irregular swellings at the anterior end of the longitudinal nerve cord. The three regions undergo much modification in the course of the development of more advanced vertebrates, showing specially thickened areas in their wall and distinctive outgrowths in other places. Despite these changes, however, the original three divisions of the brain can still be recognized even in the most advanced vertebrates, including man. The three divisions are the forebrain, midbrain, and the hindbrain.

The central canal of the spinal cord extends into the brain as a series of hollow compartments or ventricles. These like the canal are filled with cerebrospinal fluid. Both the brain and spinal cord are wrapped in protective membranes known as meninges. The spaces between the three meninges are filled with cerebrospinal fluid which acts as a cushion that prevents damage to the nervous tissue by the bones in which they are encased. Very early in its evolution the brain underwent modifications that set the stage for later evolutionary trends.

The mechanism of the modification, which we now term morphogenesis, has puzzled and challenged developmental biologists for the past eighty-five years. The majority of the studies within the past seven years have their emphasis based on cell migration, cell interaction, and more recently on nucleocytoplasmic interactions (Browne, 1970b).

Cells and tissues do not differentiate in a vacuum; yet the importance of the surrounding and internal fluids and morphogenesis has remained relatively neglected. Nevertheless, some results have shown that the ontogeny of embryonic fluids can be studied and that they are important to the orderly changes in cellular aggregates we call morphogenesis.

According to Bell and Freeman (1971), there is a dearth of information concerning the extravascular fluids of the domestic fowl. No doubt the difficulties of sampling fluid like the cerebrospinal fluid account, at least in part, for the situation. A survey of the literature has failed to generate information on the synovial fluid, the extravascular, interstitial fluid, or the water content of the skeletal system.

The cerebrospinal fluid is thought by some to be an ultrafiltrate or dialysate of the blood plasma (Flexner, 1938); by others, to be a secretion involving energy expenditures by the cells of the choroid plexus. Flexner has shown that the cerebrospinal fluid changes from an ultrafiltrate to a secretion during sequential development of the fetal pig.

The cerebrospinal fluid, as first described by Magendie in 1825 (cited in Weed, 1922), is a clear limpid liquid of low specific gravity (1.004-1.006), colorless, and of a slight but definite viscosity. When withdrawn during life, according to Weed (1922), the liquid usually contains noticeably sparse cell populations per cubic millimeter (less than 10), but in many pathological conditions its cell content may be enormously increased.

Work presented by Browne (1970b) has shown that there are marked changes in the osmotic pressure of the cerebrospinal fluid (CSF) of the normal chick embryo during the process of development. The osmotic pressure of normal cerebrospinal fluid increased from 288 milliosmols on day 4 to 351 milliosmols on day 8. These changes in cerebrospinal fluid provide a possible mechanism for the expansion of the neural tube which occurs during days 4 through 7. High osmotic pressure leads to fluid absorption and increased cerebrospinal fluid volume which in turn leads to brain expansion.

Moreover, when dimethyl sulfoxide (DMSO) was used as a source of physiological insult in chick embryos, noticeable increases in osmotic pressure and, as a consequence, brain expansion, led to mid-brain ruptures (Browne, 1970b). In this present study, the overall effects on brain morphogenesis via viscosity of mid-brain cell secretions were investigated.

The purpose of this study was to determine whether there is any possible correlation between the osmotic pressure changes in the CSF of the normal developing chick embryo and changes in the viscosity of the fluid at this time interval, and to observe and determine the effects of DMSO on CSF viscosity.

CHAPTER II

REVIEW OF LITERATURE

The cerebrospinal fluid, as first effectively described by Magendie in 1825 (according to Weed, 1922), is a clear limpid liquid of low specific gravity (1.004-1.006), colorless, having a slight but definite viscosity. Bell and Freeman (1971) reported that Anderson and Hazelwood found the specific gravity of cerebrospinal fluid to be 1.007, which is in accordance with values obtained by Magendie.

Various estimates of the amount of fluid existing in the cerebral ventricles and around the nervous system in adult man have been reported. The computation of 100 to 150 g given by Testus in 1905 (see Weed, 1922), has been the most reliable. However, because of the complexities of the fluid bed, the figure is utilized only as an approximation. According to Anderson and Hazelwood, as related in Bell and Freeman (1971), cerebrospinal fluid is extremely difficult to collect from birds and only approximately 0.5 ml may be obtained from birds older than 2 months.

Chemical analyses have demonstrated that the cerebrospinal fluid contains small quantities of inorganic salts bound to proteins and dextrose. The inorganic salts are chiefly NaCl and KCl, which occur in ratios of 17.3 to 1 according to Mestrezat (1912). Anderson and Hazelwood and Wilson, Kigrle, and Glick (as related in Bell and Freeman, 1971), have shown that the mean concentration of sodium is 159 mEg/L; potassium is 4.16 mEg/L and chloride 142 mEg/L. The average pH value was found by Felton, Hussey, and Baqne-Jones in 1917

(see Bell and Freeman, 1971), to be 7.75 with maximum variations of 7.4 and 7.9. These type chemical and physical characteristics may have promoted Halleburton to describe cerebrospinal fluid (CSF) as "an ideal physiological saline solution" bathing the neurons and maintaining their osmotic equilibrium (see Weed, 1922). Evidence points to a constant production of the cerebrospinal fluid, its passage through the cerebral ventricles and thence throughout the subarachnoid space, and its subsequent major absorption into the nervous system.

The cerebrospinal fluids was thought to be an ultrafiltrate or dialysate of the blood plasma (Fremont-Smith, 1927; Becht and Matill, 1920; Foley, 1923; Mestrezat, 1912). Bering (1955, 1962) has suggested that cerebrospinal fluid is a secretion involving energy expenditures by the cells of the choroid plexus. Flexner (1938) has shown it to change from an ultrafiltrate to a secretion in the fetal life of the pig.

A number of investigators has studied the alterations in pressure of the CSF affected by the introduction of various substances into the blood stream or alimentary canal. Accompanying these changes in fluid pressure, Weed and McKibben found marked alterations in the volume of the brain (see Weed, 1922). The hypertonic solution produced a small shrunken brain, while the hypotonic solution caused marked swelling of the brain substance. The experimental changes in brain volume were particularly pronounced in animals in which the cranial cavity had been opened by trephining.

Browne (1970a) has shown that there are marked changes in the osmotic pressure of the CSF of the normal chick embryo, during development. Dimethyl-sulfoxide has been shown to increase the osmotic

pressure of cerebrospinal fluid (Browne, 1970b). This also agrees with the work of Dixon and Halleburton who found that drugs caused a secretory rise in CSF pressure. They declared that the CSF serves as the lymph of the brain and seems to function as an accessory fluid to the central nervous system (see Weed, 1922).

In a study of mylboshistic human fetuses by Chaube and Swingyard (1975), the CSF seems to communicate directly with the amniotic fluid for at least 3 to 4 weeks. This implies that CSF is probably a principal source of increased alpha fetoprotein concentration encountered in amniotic fluid of all pregnancies with neural tube defects. When biological variables are recognized, it is evident that increased concentrations of amniotic fluid alpha protein is a reliable indicator of fetuses with open myelocele and/or anencephalus.

Development of pia and arachnoidal membranes in the mouse occurs in 4 stages: The first (parental days 10-13) follows closure of the neural tube and is a period of initial vascularization of the developing telencephalon. The mesenchyme, over the telencephalon surface in the 10-day fetus has a typical large extracellular space. According to McLone and Bondareff (1975), by the 13th fetal day CSF begins to seep into and replace this extracellular space.

In handling the normal cerebrospinal fluid and drug-induced CSF, Browne (1970b) noticed a change in the viscosity of this fluid. He gave an empirical postulation that at 4 days the cerebrospinal fluid of the chick embryo is extremely viscous, practically a gel. It decreased in viscosity at successive 24-hour periods on days 5 through 7. On day 8 cerebrospinal fluid viscosity increased, but still was not quite as mucilaginous as on day 4.

Grabowski (1970) noted that the cerebrospinal fluid of young chick embryos stained with Alcian blue indicated the presence of acid mucopolysaccharides. On this basis Browne (1970b) concluded that the viscosity changes suggested that possibly the osmotic pressure changes may be due to regulated degradation of mucopolysaccharides.

The rate of cerebrospinal fluid formation in man has been studied by Rubin, Henderson, Walker and Rall (1966). Using an inulin dilution technique, they estimated the rate of cerebrospinal fluid formation to be 0.37±0.1SD ml/min. This value compares favorably with other estimates in the literature (Tubiana, Benda, and Constans, 1951; Davson, 1967; Davson and Segal, 1971), and represents a formation rate of 500 ml/day. Since the total cerebrospinal fluid volume in man is approximately 140 ml (Last and Tompsett, 1953), the cerebrospinal fluid volume is probably renewed every 6-8 hr.

The cerebrospinal fluid system, according to Milhorat (1972), is hydrostatically precise and is responsive to a wide variety of physiological influences. Ayers (1924) reported that there are several factors which determine the cerebrospinal fluid pressure: (1) The "secretion pressure" of the cerebrospinal fluid, (2) the rate of absorption, (3) the intracranial arterial pressure, (4) the intracanial venous pressure, (5) the bulk of the brain, (6) the hydrostatic pressure, and (7) the pressure of the surrounding coverings (dura and skull). Of these, the influence of the transmitted venous pressure is the most important (Becht, 1920; Bowsher, 1960; Davson, 1967). The cerebrospinal fluid pressure is increased by vasodilatory influences such as hypercapnia, hypoxia, and inhalation anesthesia (this initially raises alveola CO_2),

and is decreased by vasoconstrictory influences such as hyperventilation and hypothermia.

From a clinical standpoint, it has been argued that the association of choroid plexus papillomas and hydrocephalus is indirect evidence of the secretory role of the choroid plexuses. Conclusive proof of cerebrospinal fluid "oversecretions" by choroid plexus papilloma has yet to be demonstrated. Cushing (1914, 1926) reported that during the course of intraventricular operations, serous fluid could frequently be seen to collect on the surface of the exposed choroid plexus. Milhorat (1972) confirmed these observations while performing open choroid plexectomies for hydrocephalus. Schaltenbrand (1955a, b) has pointed to the condition of hydranencephaly as evidence of choroid plexus secretion. It is certain that a considerable volume of CSF is formed constantly in the cerebral ventricles to be absorbed mainly within the subarachnoid space. This fact was firmly established by Dandy and Blackfan in 1914 following a series of classical experiments in which they successfully produced hydrocephalus in dogs by obstructing the aqueduct of Sylvius. It is now clear that any obstruction along the cerebrospinal fluid pathways from the lateral ventricles to the subarachnoid space will result in ventricular enlargement proximal to the point of obstruction.

CHAPTER III

MATERIALS AND METHODS

Eggs for the experiment were of White Leghorn stock. They were incubated in a forced draft incubator, maintained at a temperature of 38 C ± 1 C. Cerebrospinal fluid samples were collected on days 4 through 8 by the following procedure. The chick embryo was removed from the yolk and surrounding membranes while submerged in 0.85%, physiological saline. The embryo was carried through three stages of cleaning. It was then placed on an absorbent pad to absorb the surrounding residual wash suspension in preparation for CSF extraction.

The CSF was extracted from the mid-brain region of the embryo by a glass microneedle, to which polytubing was attached, making a pipette in the manner described by Browne (1970b) with some modification by McMillan (1975). The CSF was transferred to a microcentrifuge tube and centrifuged in a Beckman-microfuge to separate cellular debris. Samples of same age embryos were separated and carefully transferred to a second microtube and 0.5 cc of the CSF placed in a Well's Brooksfield-microviscometer. This viscometer has a constant temperature of 37 C and a spindle range of 25.70 cps. Its testing speed was set at 12 rpm. After reading, the sample was removed and pipetted back into the microtubes.

The dimethyl-sulfoxide (DMSO), obtained from Crown-Zellerbach Corporation and used in this study, was classified as animal grade (high purity), i.e., 99.6% DMSO and 0.4% water. The DMSO was diluted to a 50% concentration with saline (0.85%).

For these experimental procedures aseptic conditions were maintained by swabbing the blunt end of the egg and the syringe with 70% alcohol. Tuberculin syringes calibrated in 0.1 ml units were used for injections. All injections were made by puncturing the blunt end of the egg and dropping the solution into the air-sac. A 0.5 ml volume was injected into the air-sac. In this investigation both saline injected and untreated embryos served as controls.

After injection, the holes in the air chambers were sealed with melted paraffin and the eggs were immediately returned to the incubator. The eggs were examined by incandescence daily and those showing signs of injury or death were removed, examined and noted. Fluid samples were taken from visibly stressed embryos to determine the viscosity. Likewise, samples of those of normal appearance at the given day were collected for viscosity determinations.

Cerebrospinal fluid samples were collected 6 to 8 hr after injection on day 4. On all other remaining days the fluid was aspirated at an even number of hours. The fluid samples were transferred to microtubes and then transferred to the Beckman-microfuge and centrifuged, as in the case of the normal CSF extractions, to get rid of cellular debris. The fluid samples were then transferred to the Well's Brookfield microviscometer to obtain the viscometer readings. The entire procedure for determining the viscosity was completed in the average time of 4 to 5 min.

A total of 2,640 embryos was used in this investigation. Of this total, 1,351 were used for normal CSF extractions and a total of 431

was used in the DMSO studies. The LD_{50} dosage allantoic injections (0.02 ml of 50% DMSO), was established by Browne (1970a).

Table 1. Total number of embryos from which cerebrospinal fluid was extracted during normal viscosity investigation.

Day	Total
4	422
5	335
6	216
7	151
8	227
Total number embryos used	1,351

Table 2. Total number of embryos used for cerebrospinal fluid extraction from 4- through 8-day normal viscosity investigation.

Day	Total
4	540
5	540
6	360
7	240
8	240
Total number embryos used	1,920

Table 3. Total number of embryos from which cerebrospinal fluid was taken after DMSO air-sac injections on day 4.

Day	Total
4	112
5	97
6	47
7	77
8	98
Total number embryos used	431

Table 4. Total number of embryos used for DMSO air-sac injections in 4- through 8-day chick embryo.

Day	Total
4	300
5	120
6	60
7	180
8	60
Total number embryos used	720

CHAPTER IV

RESULTS

Normal Viscosity Changes in Cerebrospinal Fluid

Our data show that at day 4 viscosity is at a marked high; from day 5 through 6 the viscosity is reduced, and at day 7 through 8 the viscosity is markedly increased. Days 4 through 8 represent a period of active expansion and compartmentalization of the brain in chick development. In handling cerebrospinal fluid samples, there are noticeable changes in the viscosity of these fluids. At day 4 the cerebrospinal fluid was found to have marked mucilaginous properties, subsequently fluctuated, becoming highly viscous by day 8. On days 5 through 7 the viscosity of cerebrospinal fluid decreased at day 6.

Viscosity calculations indicate that the cerebrospinal fluid had an average range of 1.1822 cps on day 4 and began decreasing thereafter to 1.2210 cps. On day 8 the average viscosity reading continued to increase to 1.2850 cps (Fig. 1, Table 3). The viscosity of distilled water was calculated to be 1.0280 cps, and this was used as a parameter for measuring changes in the analyzed cerebrospinal fluid.

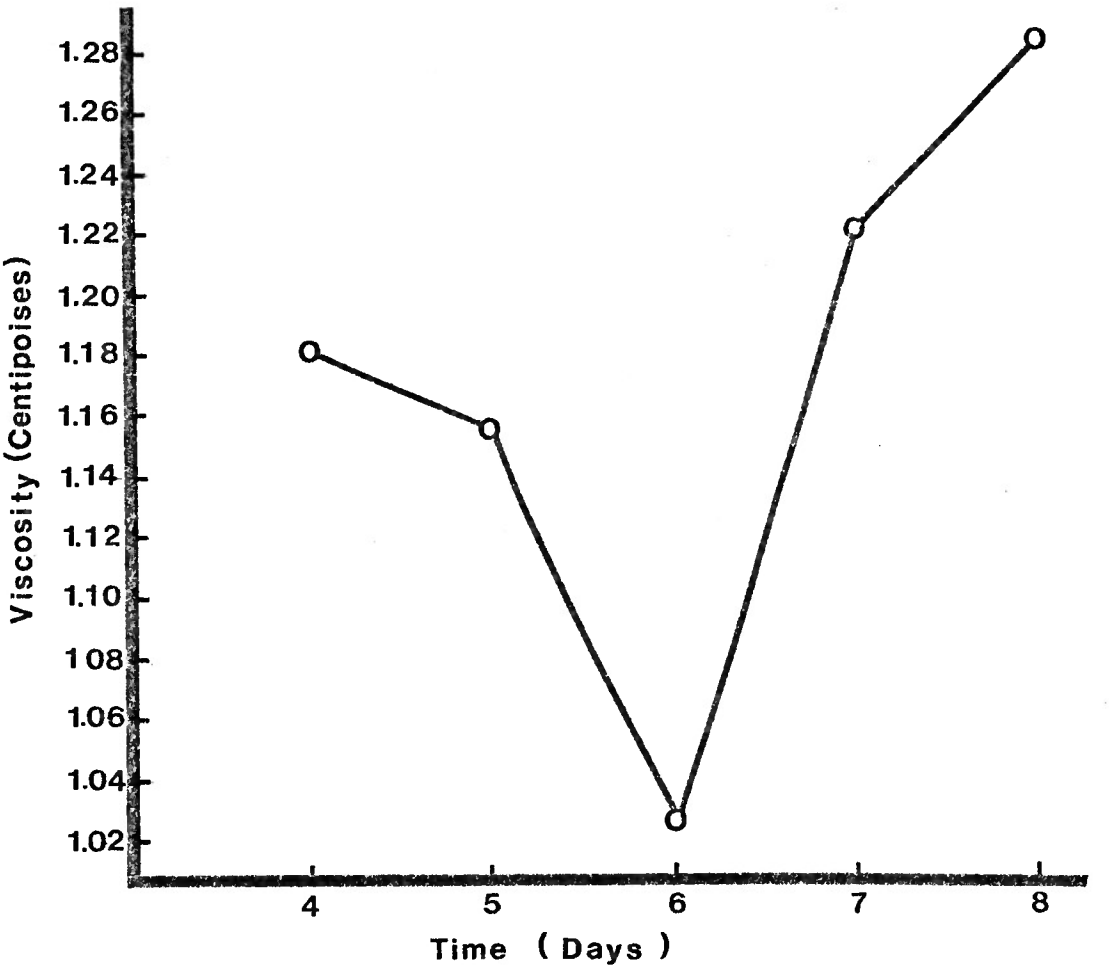

Viscosity (Centipoises)
1.28
1.26
1.24
1.22
1.20
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
4
5
6
7
8
Time (Days)

Table 5. Changes in viscosity of normal cerebrospinal fluid in 4- through 8-day chick embryos.

Days	Viscometer reading	Calculated viscosity (cps)
4	4.60	1.1822
5	4.50	1.1565
6	4.00	1.0280
7	4.75	1.2210
8	5.00	1.2850
Control distilled water	4.00	1.0280

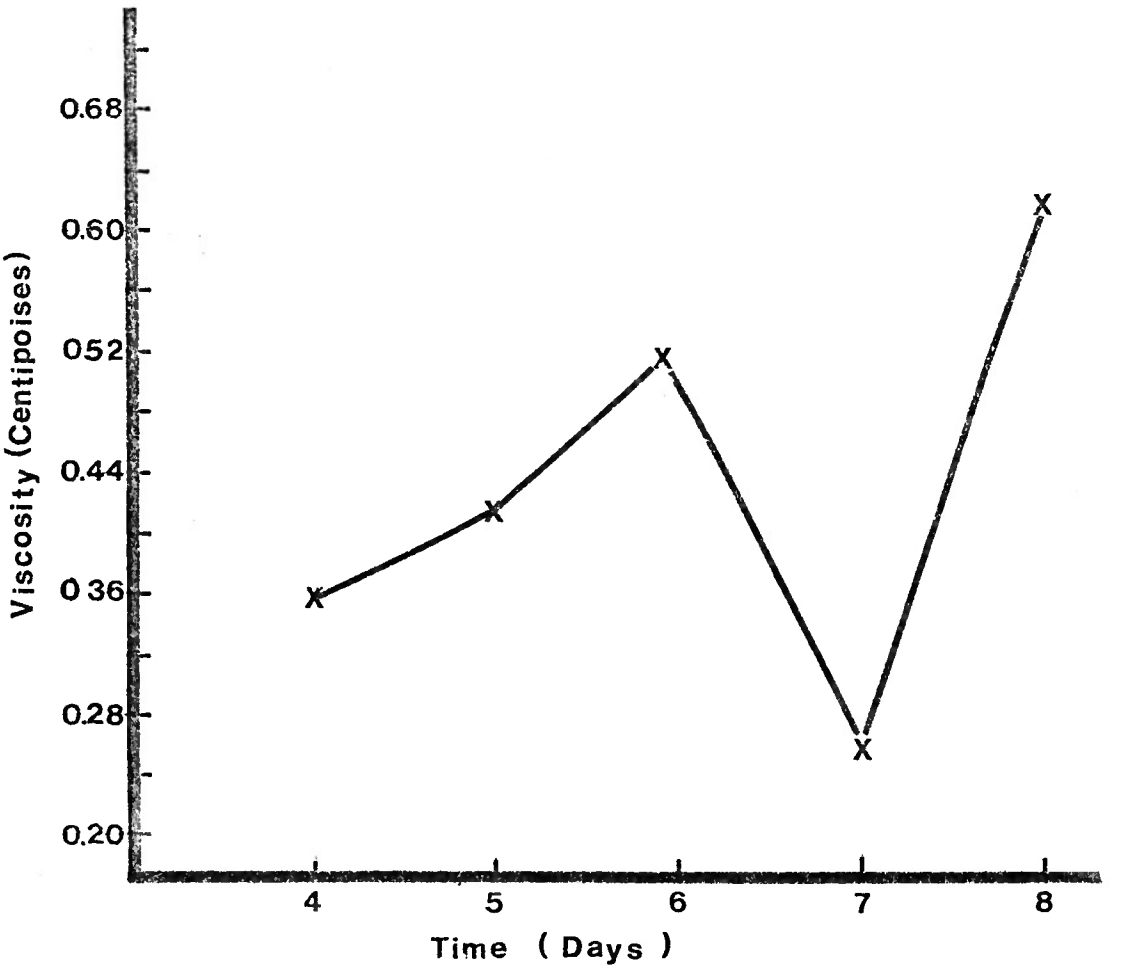
Effects of Dimethyl-sulfoxide on Viscosity of Cerebrospinal Fluid

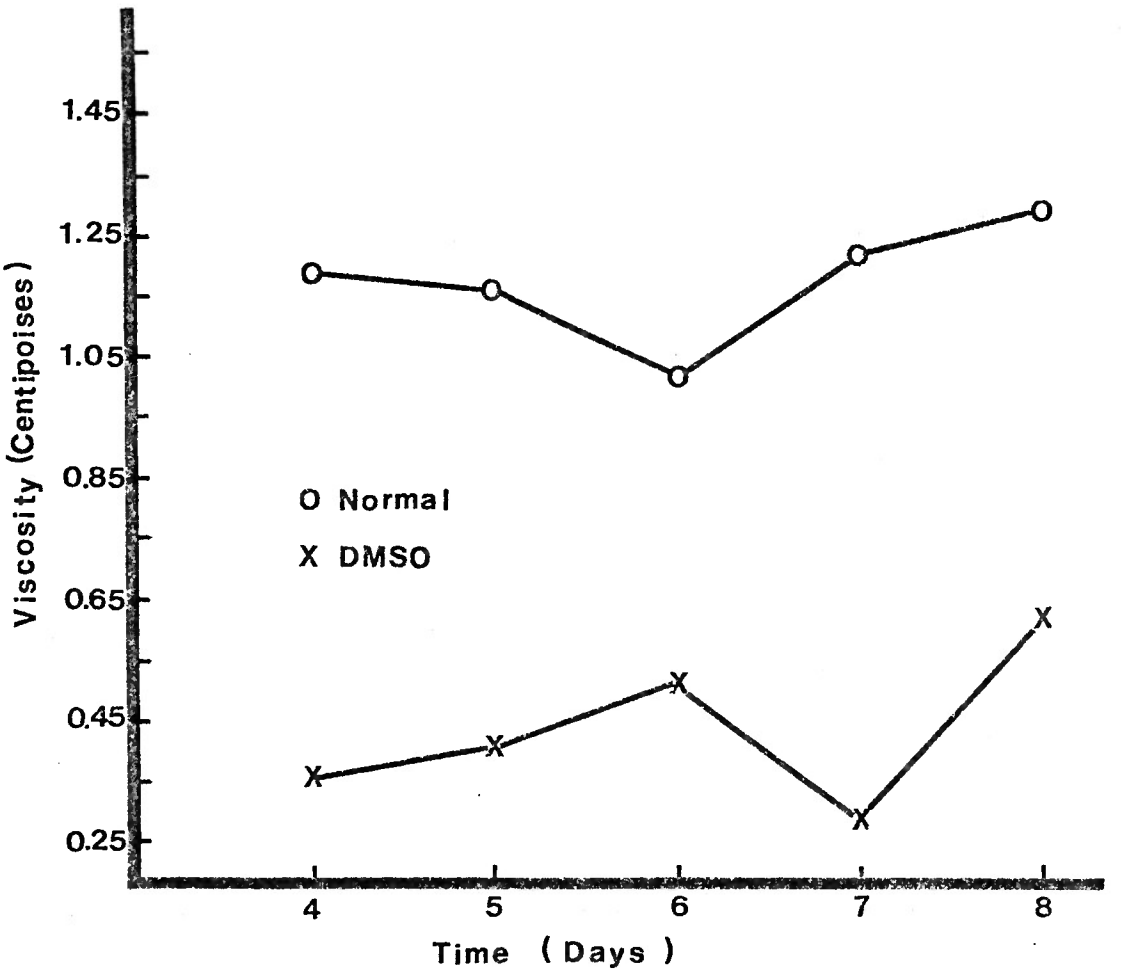
The data show that the viscosity of cerebrospinal fluid is lowered considerably at day 4 following administration of 0.5cc DMSO. The eggs were examined 6 to 8 hr after DMSO treatment on day 4. The viscosity calculated in centipoise (cps) gave a reading of 0.3598 cps, which was considerably lower than the viscosity of normal cerebrospinal fluid at day 4. By day 6 the viscosity had reached a maximum value of 0.5140 cps, which was still lower than day 6 of the normal cerebrospinal fluid viscosity. From day 6 through 7 it began to taper off, with a marked decrease by day 7. By day 8 the viscosity of the cerebrospinal fluid was markedly increased to 0.6170 cps (Table 6, Fig. 2).

The results of these experiments show that DMSO decreases the normal viscousness of cerebrospinal fluid, but graphically they are inversely proportional up to day 7. On day 8 there was the noted increase in viscosity (Fig. 3). The critical point of interest occurs at day 6 in which the maximum viscosity value with the administration of DMSO is 0.5140 cps, and the minimum viscosity of normal cerebrospinal fluid occurs also at day 6. Observations show that between days 4 and 6 the volume of fluid collected was more than that of normal viscosity. The fluid collected on day 4 and 8 was not as viscous as was seen on days 4 and 8 of the normal cerebrospinal fluid. The mid-brain regions of the chick embryo, where DMSO was administered, showed signs of a greater outpocketing of the mid-brain region. Those embryos which were morbid showed signs of blood blisters and/or ruptured mid-brain walls.

Table 6. Changes in viscosity of cerebrospinal fluid in 4- through 8-day chick embryos after DMSO air-sac injections on day 4.

Day	Viscometer reading	Calculated viscosity (cps)
4	1.4	0.3598
5	1.6	0.4112
6	2.0	0.5140
7	1.0	0.2570
8	2.4	0.6170


Viscosity (Centipoises)
0.68
0.60
052
0.44
0 36
0.28
0.20
4
5
6
7
8
Time (Days)


Viscosity (Centipoises)
1.45
1.25
1.05
0.85
0.65
0.45
0.25
O Normal
X DMSO
4
5
6
7
8
Time (Days)

CHAPTER V

DISCUSSION

It has been more than three centuries since William Harvey (1628) accurately described the circulation of the blood in his De Motu Cordis, and the information on the physiology of circulation of the cerebrospinal fluid still remains incomplete. Data regarding the formation and absorption of the cerebrospinal fluid are especially almost nonexistent. Of the information that is available, conflicting data and widely opposing viewpoints are numerous. In the present investigation, changes in the viscosity of mid-brain cell secretions in the chick embryo are reported.

In this investigation, it was found that there are marked changes in the viscosity of cerebrospinal fluid between days 4 through 8 of incubation in the chick embryo. The data show that at day 4 viscosity is markedly high, as compared to distilled water. From days 5 through 6 the experimentally determined viscosity of the chick embryo cerebrospinal fluid is reduced and at day 7 is markedly increased and continues increasing through day 8. Since there is a marked change in the viscosity of the cerebrospinal fluid from days 5 through 6, it is suggested that this change may be due to changes in the macromolecular composition of the cerebrospinal fluid in a regulatory sequence of timed intervals.

From these present and previously reported studies (Weiss, 1934; Browne, 1970a), it is apparent that the cells of the embryonic brain

of chick plays some influential role in regulating these changes. It has been shown by Weiss (1934) that the inner layer (ependymal cells) of the embryonic mid-brain of the chick conducts secretory activity. Cerebrospinal fluid appears to be formed at the extrachoroidal sites.

Regardless of its origin, the formation of extrachoroidal fluid is of clinical interest and suggests a possible explanation for the frequent failure of choroid plexectomy as a clinical treatment for hydrocephalus. With the suggested secretory role of the brain cells, it is proposed that there are macromolecules at the cell surface which are undergoing structural changes.

The use of dimethyl sulfoxide (DMSO) has been proposed for many important therapeutic measures in man, animals and plants. Many claim DMSO is safe; others have shown it to be toxic. In the second part of this investigation DMSO was used to compare the normal viscosity changes in the 4- through 8-day chick embryo with that of possible drug-induced viscosity changes.

In a previous study (Browne, 1970a), it was found that DMSO administered into the allantois of the 4-day chick embryo produced drastic changes in salt and fluid content of the blood serum and in the allantoic and amniotic fluid compartments. Browne (1970b) reported that DMSO produced marked changes in the volume and viscosity of the cerebrospinal fluid. These changes can probably be correlated with abnormal development induced by the presence of DMSO. From all indications, in our present investigation DMSO seems to decrease the

concentration of mucin producing molecules in cerebrospinal fluid in the 4- through 8-day chick embryo as compared with that of normal cerebrospinal fluid decreasing at this period. The ease of aspiration of normal cerebrospinal fluid compared to that of DMSO-treated embryos leads us to make this postulation. As compared to the normal viscosity studies, the results of the DMSO-treated embryos yield viscosity changes which are inversely proportional to normal viscosity changes up to days 7 through 8, at which time the viscosity undergoes a striking increase.

As stated earlier, Browne (1970a) showed that cerebrospinal fluid of the chick embryo on days 4 through 7 undergoes considerable increases in osmotic pressure. The present study on the effects of DMSO on viscosity changes confirm these previous results, since increases in viscosity can cause increases in osmotic pressure. The results obtained by Browne (1970b) differ in that the increase is continued up to day 7 in osmotic pressure studies. In our present viscosity studies the increases occur up to day 6, subsequently decreasing on day 7 and abruptly and strikingly increasing again on day 8.

Flexner (1938) compared the composition of the plasma and the cerebrospinal fluid and found that the cerebrospinal fluid contained higher concentrations of magnesium and chloride ions and a lower concentration of glucose, proteins, amino acids, uric acid, calcium, phosphate, and pottasium ions. As mentioned earlier in this paper, these findings, and the energy calculated to created these disparities,

suggested that the cerebrospinal fluid is formed by active secretion, and entirely as an ultrafiltrate. The present study confirms such results in that increases noted in volume of cerebrospinal fluid were found in normal and DMSO viscosity studies. The work of Browne (1970b) also agrees with this condition, since he also noted volume changes in CSF.

Weiss (1934) has shown that there is a secretory activity of the inner layer (ependymal cells) of the embryonic mid-brain of the chick, as revealed by tissue culture. Also the studies of Milhorat et al. (1971) along with Dandy and Blackfan (1914), indicate that a considerable volume of the cerebrospinal fluid is formed constantly in the cerebral ventricles, as shown in bilaterally plexectomized and hydrocephaly investigations, respectively.

The cerebrospinal fluid system consists of a series of interconnecting chambers that support, surround, and protect the neural elements. The system is hydrostatically precise and is responsive to a wide variety of physiological influences. The previously observed morphological changes seem to proceed changes in fluid activities, namely osmotic pressure and viscosity. We suggest that these changes may be the underlying basis for expansion of the hollow tube of the brain during this period.

CHAPTER VI

SUMMARY AND CONCLUSION

Cerebrospinal fluid is thought to be an ultrafiltrate of dialysate of the blood plasma and/or a secretion involving energy expenditures by the cells of the choroid plexus. The ontogeny and physiological role in embryogenesis, a priori, of the brain is not known. This present study has advanced the postulations that regulatory changes in viscosity play a critical role in the orderly physiological changes underlying brain morphogenesis in the chick embryo.

Our studies show that: (1) The viscosity of normal cerebrospinal fluid is markedly high on day 4, with a continued decrease up to day 6; on day 7 there was an abrupt increase through day 8. (2) The mucilaginous characteristics of the cerebrospinal fluid of DMSO-treated embryos were empirically less than those of normal viscosity on days 4 through 8. (3) The volume of cerebrospinal fluid in DMSO-treated embryos is more than that of normal cerebrospinal fluid volume on days 4 through 8. (4) In DMSO studies the viscosity for day 4 was markedly low and continued to increase through day 6. At day 7 the viscosity had decreased significantly, followed by a striking and abrupt decrease from days 7 through 8.

In conclusion, we suggest that these changes in viscosity, along with previously reported osmotic pressure changes, may be the underlying cause which leads to brain cavern expansion in normal

brain morphogenesis. In the extreme cases such as DMSO-treated embryos, there is rupture of mid-brain walls and other anomalies such as hydrocephalus which have been reported in the literature.

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